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Title: Progress of Anaerobic capability at LANL

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BIOENERGY TECHNOLOGIES OFFICE

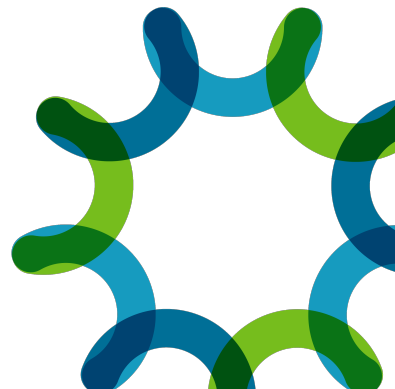
Progress of anaerobic capability at LANL

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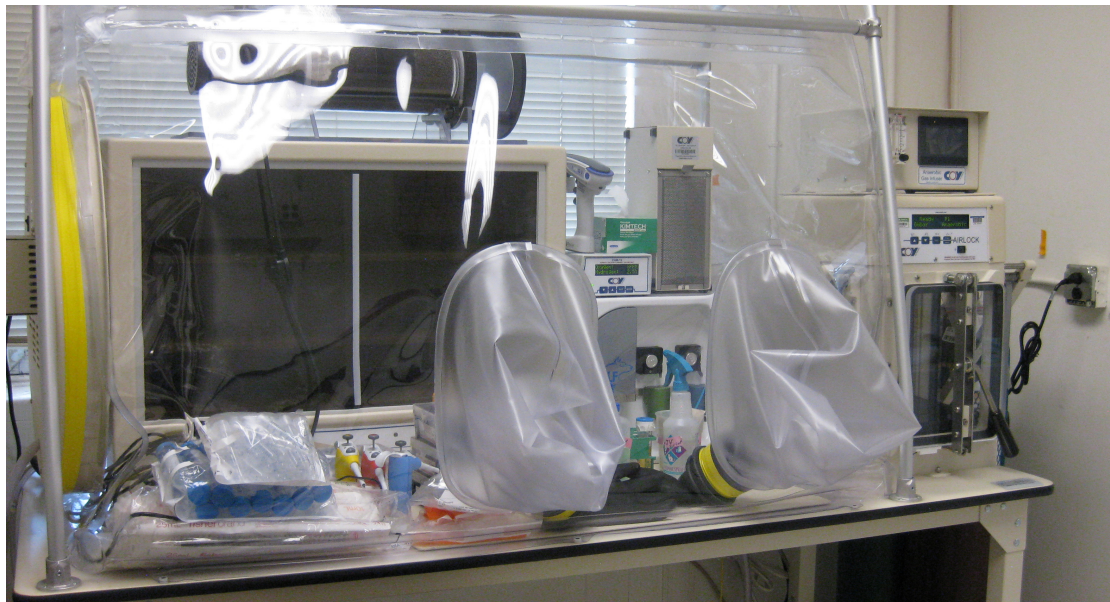
HOD

02/01/22/Los Alamos, NM



Coy vinyl anaerobic chamber set up at LANL

- Status: Anaerobic chamber is fully operational



- Anaerobic chamber provide a strict anaerobic atmosphere



Nitrogen and gas mix (5 % Hydrogen, 10 % CO₂ and 85 % Nitrogen)

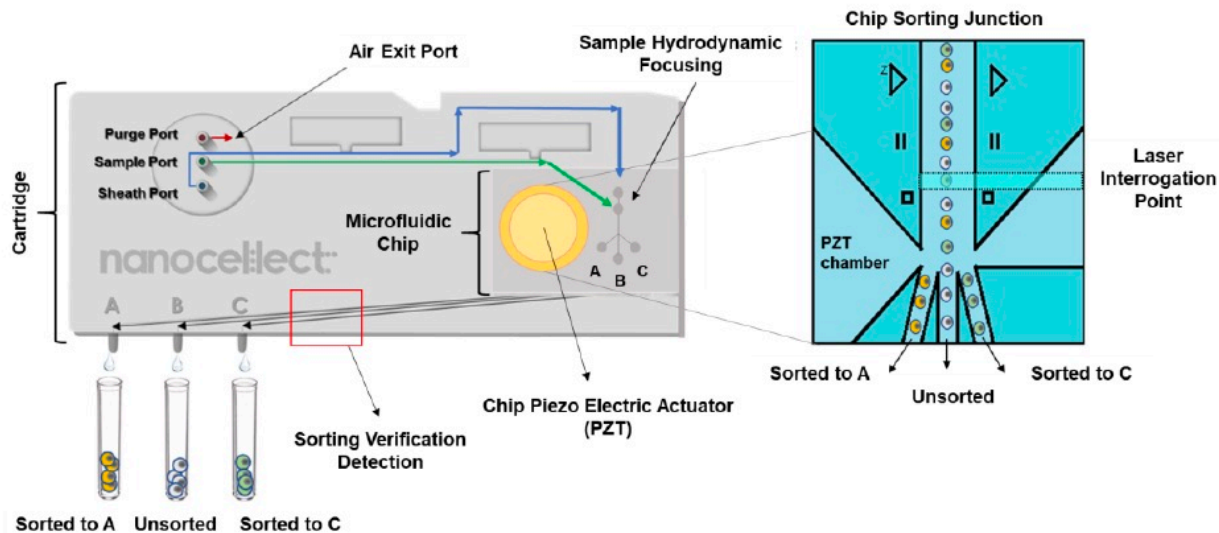
- Anaerobes are important

Wolf benchtop cell sorter set up inside the anaerobic chamber at LANL

The WOLF© Platform: Inside the Cell Sorter

5 Detection Parameters

- FSC } scatter
- BSC } scatter
- FL1 } fluorescence
- FL2 } fluorescence
- FL3 } fluorescence

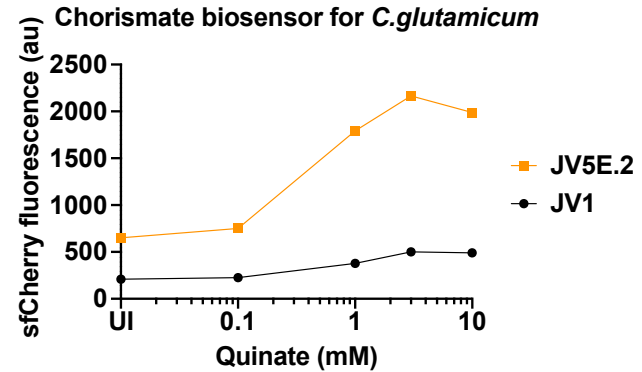
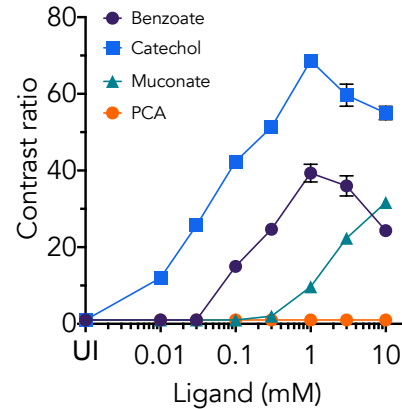
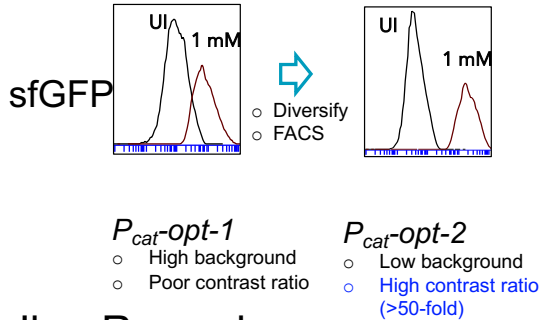


Aim: developing biosensor in anaerobes

- Currently working *with C.glutamicum*: a facultative anaerobic organism
 - We have developed muconate (CatM_sfGFP) and chorismate biosensor (QsuR_sfCherry) and dual sensing (work in progress) for *C. glutamicum*.
 - sfGFP and sfCherry need oxygen! to be able to mature the fluorescence
- For anaerobic work we are using Yfast reporter

Developing biosensor for *C. glutamicum*

C. glutamicum $\Delta catB$

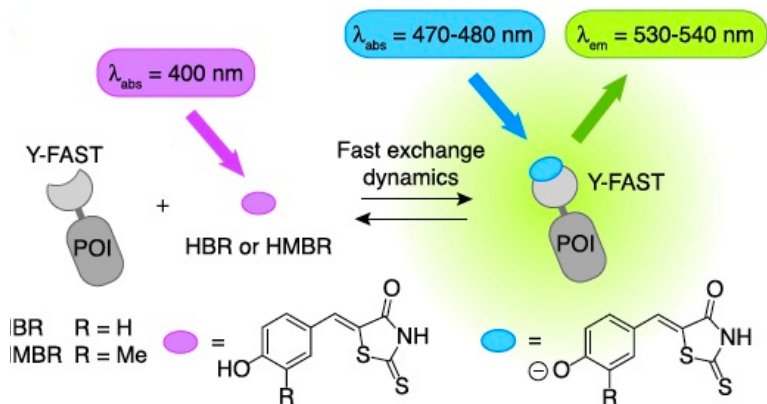


Jha, Ramesh

- Muconate sensor established in *P. putida* showed weak response in *C. glutamicum*
- Build large promoter library (~35000 diversity); FACS to select top performers
- **Outcome:** Optimized sensor with >50-fold response over baseline in new host; DBTL efficiency gain 6x
- Quinate feeds into shikimate pathway, hence can increase intracellular chorismate pool; Quinate transporter available in *C. glutamicum*
- **Outcome:** Established chorismate sensor in *C. glutamicum*. Promoter library one of the largest in *C. glutamicum*

YFAST reporter for anaerobes

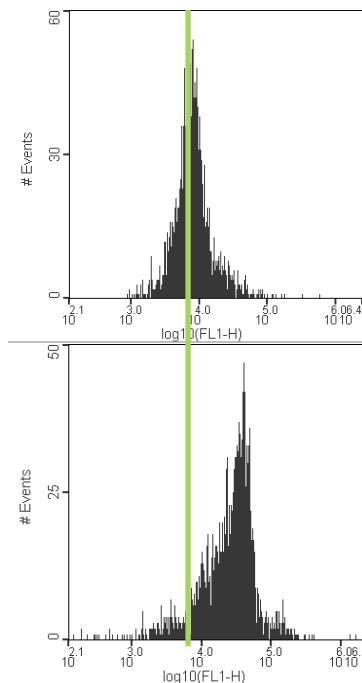
- **Yellow Fluorescence-Activating and absorption-Shifting Tag** is a 14 kDa protein tag giving a bright **green-yellow** fluorescent complex upon interaction with the **fluorogenic dye** :
- 4-hydroxy-3-methylbenzylidene rhodanine (HMBR).



Plamont M-A. 2016. *Proc.Natl.Acad.Sci.* 113 (3)
497-502

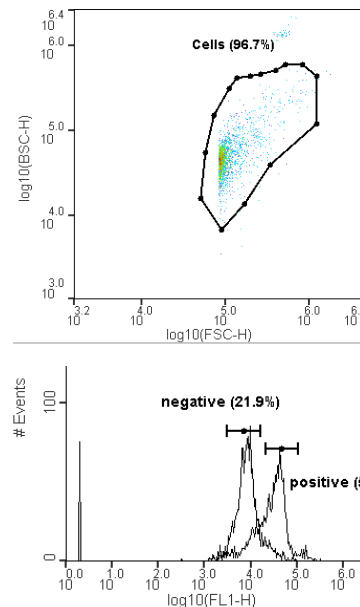
Clostridium tyrobutyricum expressing Yfast

- AG7438 (pMTV421 [*C.thermocellum* P1194-Y-FAST] in AG4492)
- Cell fluorescence was analyzed with **Wolf**



- ligand

+ ligand

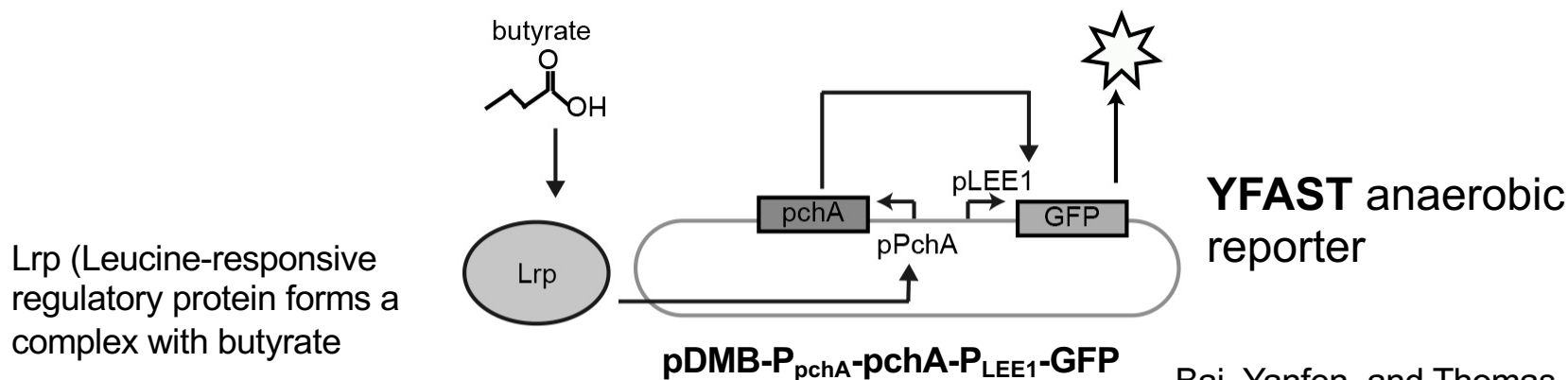


C.tyro (AG7438)
expressing Yfast +
Yfast ligand (HMBR-
3OM)

Wolf is able to detect fluorescent cells after
ligand was added to the cells

Plan: developing biosensor in *Clostridium tyrobutyricum*

- Biosensor to detect **butyric acid**



Lrp (Leucine-responsive regulatory protein forms a complex with butyrate

LEE1 (Locus of enterocyte effacement

Bai, Yanfen, and Thomas J. Mansell (2020). **Production and Sensing of Butyrate in a Probiotic *E. coli* Strain**. International Journal of Molecular Sciences, 21: 3615,

Conclusion and next steps

- Anaerobic chamber at LANL is fully functional
- Wolf cell sorter is also functional and easy to use
- *Clostridium tyrobutyricum* strains are growing at anaerobic conditions
- Biosensors in *Corynebacterium glutamicum* has been developed with aerobic reporters
- Biosensor for *C.tyrobutyricum* with Yfast will be developed soon

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LANL

- Chris Yeager
- Taraka Dale
- Ramesh Jha
- Ellin-Kristina Triolla
- Katie Wozniak
- Bert Huttanus
- Tari Kern

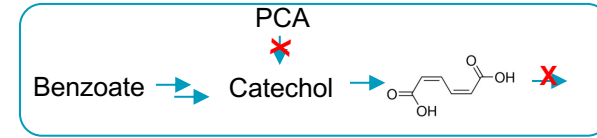
ORNL

- Adam Guss
- Melissa Tumen-Velasquez
- Austin Carroll

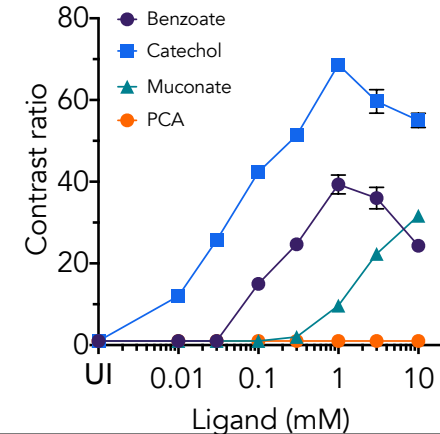
NREL

- Ray Henson
- Chris Johnson
- Gregg Beckham

Tool transfer: Establish muconate sensor in *C. glutamicum*



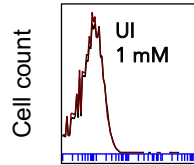
Dose Response



CatM (*A. baylyi* ADP1)



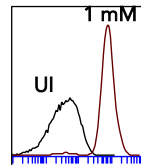
P. putida KT2440 Δ catRBC



$P_{cat-native}$
 ○ No response



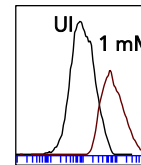
- Diversify
- FACS



$P_{cat-opt-1}$
 ○ Low background
 ○ High contrast ratio (>50-fold)



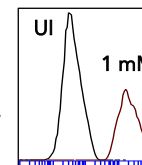
- Direct transfer



$P_{cat-opt-1}$
 ○ High background
 ○ Poor contrast ratio



- Diversify
- FACS



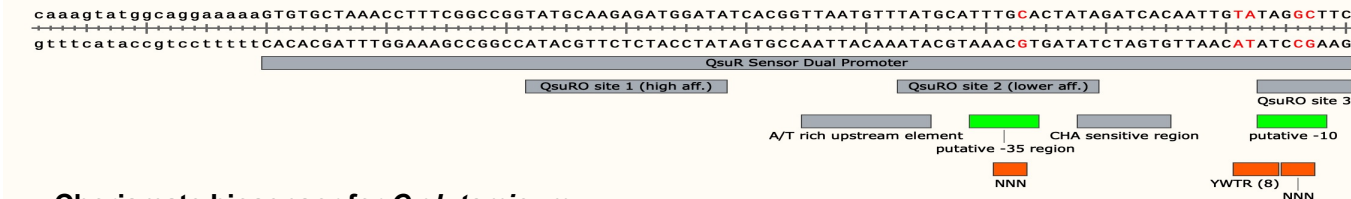
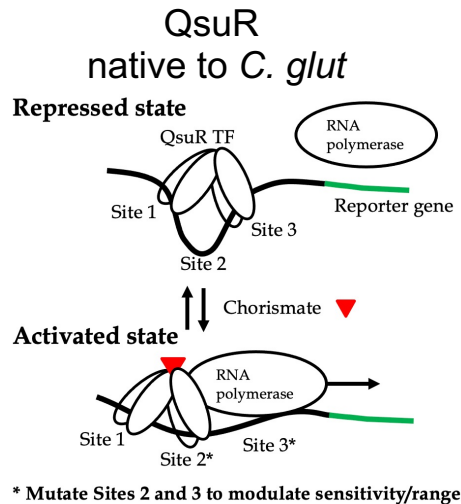
$P_{cat-opt-2}$
 ○ Low background
 ○ High contrast ratio (>50-fold)

Bentley et al, Metabolic Eng, 2020

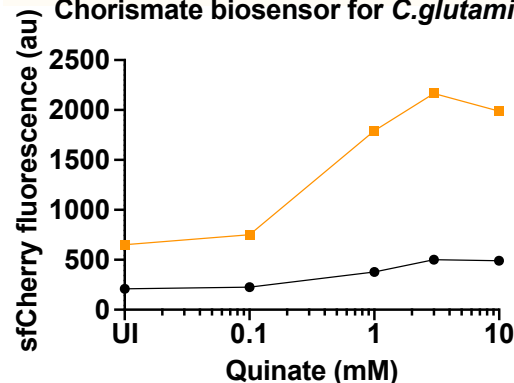
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Outcome: Optimized sensor with >50-fold response over baseline in new host; DBTL efficiency gain 6x

Development of a novel chorismate sensor in *C. glutamicum*



Chorismate biosensor for *C. glutamicum*



— JV5E.2
— JV1

4 rounds of FACS

Promotor diversification
Intended library size ~260,000
Achieved ~80%

- Quinate feeds into shikimate pathway, hence can increase intracellular chorismate pool; Quinate transporter available in *C. glutamicum*
- Outcome:** Established chorismate sensor in *C. glutamicum*. Promoter library one of the largest in *C. glutamicum*